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EXAMINER	
WOODWARD, M	

ART UNIT	PAPER NUMBER
1813	17

18N1/0324
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DATE MAILED: 03/24/94

This is a communication from the examiner in charge of your application.
COMMISSIONER OF PATENTS AND TRADEMARKS

12/13/93
12/2/93

☒ This application has been examined ☒ Responsive to communication filed on 11/26/93 ☐ This action is made final.

A shortened statutory period for response to this action is set to expire 3 month(s), 0 days from the date of this letter.
Failure to respond within the period for response will cause the application to become abandoned. 35 U.S.C. 133

Part I THE FOLLOWING ATTACHMENT(S) ARE PART OF THIS ACTION:

1. ☒ Notice of References Cited by Examiner, PTO-892.
2. ☐ Notice of Draftsman's Patent Drawing Review, PTO-948.
3. ☐ Notice of Art Cited by Applicant, PTO-1449.
4. ☐ Notice of Informal Patent Application, PTO-152.
5. ☐ Information on How to Effect Drawing Changes, PTO-1474.
6. ☐ _____.

Part II SUMMARY OF ACTION

1. ☒ Claims 12-28 are pending in the application.
   Of the above, claims _____ are withdrawn from consideration.
2. ☒ Claims 1-11 have been cancelled.
3. ☐ Claims _____ are allowed.
4. ☒ Claims 12-28 are rejected.
5. ☐ Claims _____ are objected to.
6. ☐ Claims _____ are subject to restriction or election requirement.
7. ☐ This application has been filed with informal drawings under 37 C.F.R. 1.85 which are acceptable for examination purposes.
8. ☐ Formal drawings are required in response to this Office action.
9. ☐ The corrected or substitute drawings have been received on _____. Under 37 C.F.R. 1.84 these drawings are ☐ acceptable; ☐ not acceptable (see explanation or Notice of Draftsman's Patent Drawing Review, PTO-948).
10. ☐ The proposed additional or substitute sheet(s) of drawings, filed on _____. has (have) been ☐ approved by the examiner; ☐ disapproved by the examiner (see explanation).
11. ☐ The proposed drawing correction, filed _____, has been ☐ approved; ☐ disapproved (see explanation).
12. ☐ Acknowledgement is made of the claim for priority under 35 U.S.C. 119. The certified copy has ☐ been received ☐ not been received ☐ been filed in parent application, serial no. _____; filed on _____.
13. ☐ Since this application apppears to be in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under Ex parte Quayle, 1935 C.D. 11; 453 O.G. 213.
14. ☐ Other

EXAMINER'S ACTION

The Berger Declaration under 37 C.F.R. § 1.132 filed January 10, 1994 is sufficient to overcome the rejection of claims 12-28 under 35 USC §103 based upon the combined teachings of Ekins and Chang and the rejection of claims 12-28 under 35 USC §112, first paragraph.

Paragraph 7 points to a feature of the Chang assay, multiple antigenic sites on the cells being bound to the surface immobilized antibody, which probably leads to a violation of the limitation that antibody be present at 0.1V/K.

The Roitt Declaration under 37 C.F.R. § 1.132 filed January 10, 1994 is sufficient to overcome the rejection of claims 12-28 under 35 USC §103 based upon the combined teachings of Ekins and Chang and the rejection of claims 12-28 under 35 USC §112, first paragraph.

Paragraph 5 points to a feature of the Chang assay, multiple antigenic sites on the cells being bound to the surface immobilized antibody, which probably leads to a violation of the limitation that antibody be present at 0.1V/K.

The following is a quotation of 35 U.S.C. § 103 which forms the basis for all obviousness rejections set forth in this Office action:

> A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.
>
> Subject matter developed by another person, which qualifies as prior art only under subsection (f) or (g) of section 102 of this title, shall not preclude patentability under this section where the subject matter and the claimed invention were, at the time the invention was made, owned by the same person or subject to an obligation of assignment to the same person.

Claims 12-28 are rejected under 35 U.S.C. § 103 as being unpatentable over Ekins (WO 84/01031) and Leaback (US Patent 5,096,807).

The instant claims are to methods, devices for practicing said methods and kits therefor wherein the amount of capture reagent for an analyte is such that an insignificant portion of the analyte present is removed from solution. In such situations the method becomes volume independent above a minimum volume. Essentially the error due to volume variation is insignificant. At what point error becomes insignificant is operationally defined; although it would appear to be true that the smaller the error the less significant.

In WO 84/01031 Ekins teaches, starting from the Law of Mass Action, that if the amount of antibody is sufficiently small that measurements of the amount of analyte bound thereto will be independent of volume (summarized in the abstract). Applicant has admitted these teachings as prior art. In the example of WO 84/01031 less than 10 fmoles of immobilized antibody with an affinity of 2×10^{10} L/M was employed with sample volumes of 0.2, 0.4 and 0.8 mL and it was stated that the measured concentration was the same for each volume within experimental error. The value of V/K for 0.2mL is 10 fmole. Ekins does not disclose over what area the antibody is immobilized nor how the amount of antibody immobilized was determined. Ekins suggests detecting bound analyte using fluorescently labelled reagents (page 5 of WO 84/01031) and a surface containing a plurality of sites.

Ekins is disclosing, by example, that for his definition of insignificant that an amount of antibody equal to V/K is acceptable and that a value of 0.25 V/K gives equivalent results. This is a four-fold decrease in the amount of antibody or four-fold increase in sample volume. If one goes back to the Law of Mass action and the binding equations in Ekins and asks is 0.25 V/K the theoretical limit below which a reliable binding curve can be obtained one finds that the answer is no. Even at 0.1 V/K and 0.01 V/K this is still true. The barrier to values of 0.1 V/K and lower values was not theoretical but practical at the time the Ekins application was published. Consider how Ekins coated the antibody. The coating procedure was such that the

antibody was distributed over a relatively large surface. In such a situation the density of antibody is low compared to the sites available for non-specific signal and the number of antibody sites with analyte bound is even lower. The barrier to lower values of V/K was technological. If one were able to shrink the area to which the antibody was applied then the signal to noise ratio would significantly improve, provided one shrinks the observation window. Consider the bottom of a well in a microtiter plate (6 mm dia) which is coated with antibody compared to the same surface in which the antibody is confined to a 1mm spot in the center. If noise is a constant per unit area then by shrinking the observation window to 1 mm in the above situation will result in a 36 fold reduction in noise.

At the time of Ekins it may not have been technically possible to construct a sample surface and a device to read signal such that amounts of antibody equal to or less than 0.1 V/K could be utilized. However, by the time of filing of the instant application this was not the case. Leaback teaches a device which can monitor a plurality of small sites simultaneously. This does not completely solve the problem because the device of Leaback does not necessarily rely on spots with closely spaced antibody.

The second central problem to be solved is coating the antibody such that for the assay it is at 0.1 V/K while at the same time is confined to small area. One way this would have been done would have been by coating the antibody on a surface at a density such that effectively a monolayer of antibody is obtained and then "cutting" out areas which contained 0.1 V/K for use in the assay.

Claims 12 and 23. It would have been obvious to a person of ordinary skill in the art at the time the invention was made to use the device of Leaback with sample volumes such that the amount of immobilized antibody was less than or equal to 0.1 V/K because the perturbation of analyte concentration as a result of binding is even less significant than the maximal amount of

antibody which Ekins teaches may be employed to obtain volume independent results and as a consequence error to changes in analyte concentration as a result of binding would be minimized.

Claims 13 and 24. One would minimize the spot size so that the density of signal would be higher so that the signal to noise ratio would be greater.

Claims 14 and 25. The minimal value of 10,000 will result from a calculation of optimal signal to noise ratio.

Claims 15 and 26. The limitation here is to using the device of claim 1 in which either the volume is ten-fold greater or the amount of antibody is ten fold less. In either event the changes lead to a further decrease in the error of the measurement. Such a decrease is an optimization step within the level of skill of the ordinary artisan. It can be accomplished because the means for detection, e.g., a fluorescence microscope, affords sufficient resolution. One of the problems attendant with using less antibody is a potential decrease in signal to noise so that a measurement above background can be made. But with the ability to localize the antibody to a discrete small spot and a device with the ability to resolve such spots the problem is resolvable.

Claims 16 and 17. Monoclonal antibodies exhibit affinities in this range and are an obvious choice for this method in view of the their suggested use by Leaback. They are also an obvious choice because the uniformity of affinity constant will afford a more uniform signal distribution between spots.

Claims 18 and 19. The device of Leaback when used as discussed above with regard to claims 14 meets these limitations.

Claim 20. Both Ekins and Leaback teach using antibodies as the immobilized binding agent.

Claim 21. Ekins suggests using fluorescently labelled compounds for detecting bound analyte.

Claim 22. By labelling the binding agent and quantifying the fractional occupancy as the ratio of analyte signal to binding agent signal one is controlling for variation in the amount of binding agent which is immobilized at a particular site. One of

ordinary skill in the art would perform the measurement in this fashion to control for the variation in coating density between spots.

Claims 26-28. It would have been obvious to a person of ordinary skill in the art at the time the invention was made to manufacture kits containing a surface with a plurality of immobilized antibodies such that less than 0.1 V/K was immobilized thereon and detectably labelled reagents capable of binding to bound analyte because such a kit would reduce the time required for analyzing a plurality of analytes and aid in reducing inter assay variability by employing standardized reagents.

Applicant has vigorously argued the teachings of Ekins. Both Roitt (¶6) and Berger (¶5 and ¶6) have addressed Ekins in virtually identical language. Both have made use of the Law of Mass Action and the binding equations of Ekins in an to persuade the examiner that one of ordinary skill in the art would not have further reduced the amount of antibody to levels of 0.1 V/K or less. Roitt goes so far as to suggest that the reduction is so intuitively impractical that one would not normally be bothered to apply the Law of Mass Action to calculate the fractional occupancy. The examiner finds this logic unpersuasive in view of the fact that Roitt utilized these very same equations from Ekins as part of the attempt to rebut the Examiner. Neither Roitt, Berger or applicant has established that the person of ordinary skill in the art would not have understood the concept of volume independent assays from the Ekins teachings and would have realized that their practicality was overcoming the signal to noise problem which would result from employing antibody dispersed over large spot size. It is applicant's position that the person of ordinary skill in the art at the time the invention was made would not have thoroughly analyzed the teachings of Ekins in view of the state of the art at the time the invention was made. This invention was made well after the publication of Ekins and at a time when the technology was more advanced. The

particular problem of theoretical assays and technological barriers is discussed in Ekins (1992) and is applicable here.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Michael Woodward whose telephone number is (703) 308-3890.

Any inquiry of a general nature or relating to the status of this application should be directed to the Group receptionist whose telephone number is (703) 308-0196.

Papers related to this application may be submitted to Group 180 by facsimile transmission. Papers should be faxed to Group 180 via the PTO Fax Center located in Crystal Mall 1. The faxing of such papers must conform with the notice published in the Official Gazette, 1096 OG 30 (November 15, 1989).

The CM1 Fax Center number is (703) 308-4227.

CHRISTINE M. NUCKER
SUPERVISORY PATENT EXAMINER
GROUP 180

Michael P. Woodward
March 21, 1994